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RESEARCH ARTICLE

# Obtention and characterization of chitosan/starch/roselle films for the preservation of perishable fruits

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## ABSTRACT

Packaging is the main source of polluting plastic waste. Out of the total volume of plastic waste worldwide, the majority corresponds to food packaging. Biodegradable films have been used in a large number of applications across industrial sectors due to their property versatility and environmental factors. There is a growing interest in the search for packaging materials from renewable sources that prove to be functional for food preservation. Among the polymers used, starch has garnered the most attention due to its abundance in nature and biodegradable quality; it is also renewable and low-cost. A methodology was established to increase the shelf life of perishable fruits (blackberry) from 7 to 14 days by adding starch, chitosan, and roselle extract. These biopolymers synergistically contribute by providing support, antibacterial, and antioxidant properties, respectively. The use of chitosan and roselle extract were key to the achievement of this work's goals, as proven by FT-IR, TGA, SEM, contact angle, and microbiological analyses. The present work focuses on the use of three elements that have not been studied together in biofilm design to preserve fruits.

**Keywords:** Chitosan, Packaging, Post harvest, Preservation

## INTRODUCTION

For decades, organic films and coatings have been used to promote the replacement and reinforcement of the natural layers of certain edible fruits to prevent the loss of moisture and components, allow for gas exchange, and provide sterility. To date, the scientific community remains interested in the development and improvement of these coating materials to preserve edible fruits (Pavlath & Orts, 2009; Liyanapathiranage, et al 2023).

Nowadays there is a growing interest in the design and use of biopolymer films, mostly because of the concern regarding the elimination of conventional oil-based plastic materials. A global concern, plastic degradation is a process that takes long periods of time, involving a critical level of irreversible damages to the environment. In contrast, organic films from renewable sources are easily degraded and constitute a viable alternative to traditional coatings, even more so if their additional contributions to packaging are considered (López-García & Jiménez-Martínez, 2015).

Native and modified starches are of special interest to the formulation of films since they are completely biodegradable, edible, and affordable (Versino et al., 2016; Apriyana et al., 2016). Still, films made only with starch have low water resistance and relative mechanical properties and antimicrobial activity (Arifin et al., 2016). Some works have managed to add plasticizers to starch films to prevent fragility and improve flexibility since plasticizers play a key role in the structure and properties of polymer films (Müller et al., 2008; Arrieta & Palencia, 2016). Various works have been found with the use of starch with applications in coatings for preservation of fruits where it is possible to obtain flexibility properties in the coatings (Oyam, W. et al, 2023; Punia Bangar, S. et al 2022).

A deacetylated derivative of chitin, chitosan is another biodegradable material existing in nature, and its qualities,

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including the formation of films with plasticity and antimicrobial capabilities, have been researched. The literature reports the use of chitosan in biofilm design to inhibit microorganisms (Záreate-Moreno, J.C. et al., 2023; Muñoz-Tébar, N. et al., 2023). Zeynep K. et al. (2017) studied the physicochemical and antimicrobial properties of chitosan films that incorporated turmeric and proved the thermal stability provided by chitosan. However, if both polymers, starch and chitosan, are used in biodegradable films, then packaging materials with improved physical, mechanical, barrier and antimicrobial characteristics would be obtained.

Roselle extract (*hibiscus sabdariffa*) has been used as antioxidant agent because the flavonoids in its composition provides antibacterial properties to biofilms (Dwivedi, M. et al., 2020; Aydin, G. & Elif Busra Zorlu, E.B., 2022).

Currently, there is a growing increase in fruit and vegetable consumption as an alternative to fast food and other food products. In response to this demand, several technologies have been researched and applied to improve food safety. Among them is the use of biopackaging using natural polymers with a high antimicrobial activity, as the chitosan–starch mix. Essential oils and antioxidant extracts have also been included to generate improved raw materials and increase the added value of the product. Although there currently are studies on the production of biofilms from native starch, chitosan, and roselle, these elements have only been used individually. Then, the scientific contribution of this work is the study of the three elements and their synergy when they are used simultaneously to improve biofilms regarding antimicrobial and biodegradable capacities, benignity to humans, and use for preservation of edible fruits.

## METHODS

### Materials and equipment

The following materials and equipment were used in this work: distilled water from QFI (Químicos Farmacéuticos Industriales SA de CV), acetic acid from Sigma-Aldrich (CAS: 64-19-7) medium molecular weight chitosan from Aldrich (CAS: 9012-76-4), ethanol from Merck (CAS: 67-17-5), a 250-mL soxhlet extractor from Kimax, a PerkinElmer FT-IR spectrophotometer (model Spectrum Two), a TA Instruments thermogravimetric analysis (TGA) and differential scanning calorimeter (model SDT Q600 DSC-TGA Standard), a scanning electron microscope from JEOL (model JSM-6010A), a goniometer for contact angle, roselle extract obtained in the Soxhlet extractor, and a heating and ultrasonic water bath from Branson (model 2800).

### Roselle (*Hibiscus sabdariffa*) extract

Roselle extract was obtained in a Soxhlet extractor by using 80 g dry roselle and 200 mL ethanol as solvent. The extraction process ran for 10 cycles, and the extract was cooled and kept in refrigeration.

### Preparation of 100 mL 1 and 1.5% chitosan w/v

To prepare chitosan at different concentrations, 1 g chitosan was placed in a 100-mL volumetric flask and 50 mL 2% acetic acid was added. The flask was placed in an ultrasonic bath and kept under constant stirring. Acetic acid was added every hour until reaching 100 mL. The solution obtained was 1% chitosan (w/v). The process was repeated to prepare 1.5% chitosan (w/v).

### Preparation of 100 mL 0.5% starch w/v

To prepare 0.5% starch, chayotextle starch (0.5 g) was placed in a 200-mL flask and 10 mL distilled water was added. The flask was heated on a hot plate magnetic stirrer at 60 °C for 2 h.

## PREPARATION OF CHITOSAN/ROSELLE (*Hibiscus sabdariffa*) EXTRACT MATRIX

### Film preparation by casting

The films were obtained by casting, where 50 mL chitosan–starch–roselle solution was added to a 250-mL flask at the ratio shown in Table 1. Each solution was magnetically stirred for 1 h, and 2mL solution was placed on a 10-cm Petri dish, which was oven dried at 45 °C for 2 h. Once the sample was dried, the film that was formed was removed from the dish and kept in sealed bags for characterization.

### Preparation of films by fruit dipping

To obtain the films by dipping, 50 mL chitosan–starch–roselle solution was placed in a 250-mL flask, according to the ratios

**Table 1: Chitosan/starch/roselle extract matrix**

| Sample | % Chitosan | % Starch | Hibiscus Extract (mL) |
|---|---|---|---|
| M1 | 1 | | |
| M2 | 1 | | 0.5 |
| M3 | 1 | | 1 |
| M4 | 1.5 | | |
| M5 | 1.5 | | 0.5 |
| M6 | 1.5 | | 1 |
| M7 | 1 | 0.5 | |
| M8 | 1 | 0.5 | 0.5 |
| M9 | 1 | 0.5 | 1 |
| M10 | 1.5 | 0.5 | |
| M11 | 1.5 | 0.5 | 0.5 |
| M12 | 1.5 | 0.5 | 1 |
| M13 | | 0.5 | |
| M14 | | 0.5 | 0.5 |
| M15 | | 0.5 | 1 |

on Table 1, and the solution was stirred on a magnetic stirrer for 1 h. The fruit was then dipped for 1 min, and left to dry at room temperature for 1 h. Finally, the fruit was refrigerated on aluminum trays at 7 °C. Table 1 shows the working matrix to obtain chitosan/starch/roselle extract films.

### Characterization

The films obtained were characterized by Fourier transform-infrared spectroscopy (FT-IR) for functional groups. A morphological analysis was applied through scanning electron microscopy (SEM), and the contact angle was studied on the films along with antimicrobial activity (fruit senescence).

### Fourier transform-infrared spectroscopy

The FT-IR analysis of the chitosan/starch/roselle extract was carried out in a spectrophotometer (model Spectrum Two, PerkinElmer); ATR system was used in the region 650–4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$.

### Scanning electron microscopy

A high-vacuum scanning electron microscope (model JSM-6010LA, JEOL) was used in the morphological analysis at 3500x, 20 kV, and gold coating.

### Contact angle analysis

The contact angle analysis was carried out on films placed on Petri dishes. Using a goniometer, one droplet (20 µL, 44% moisture) at 25 °C was placed on the film, an image was recorded, and the angles formed by the droplet were measured. The analysis was done in triplicate and the mean was obtained.

### Thermogravimetric analysis

A differential scanning calorimeter (TA Instruments, model SDT Q600 DSC-TGA Standard) was used to carry out the TGA. The samples ranged from 1 to 3.65 mg, and the process started at room temperature until reaching a maximum temperature of ~780 °C.

### Senescence analysis of blackberries

The antimicrobial activity and fruit senescence (weight loss) of the chitosan/starch/roselle extract films was evaluated. To do so, the blackberries were coated with a film according to the matrix previously described.

The fruits were coated by dipping for 1 min and were then air dried at room temperature. The coated blackberries were placed on trays and refrigerated at 7 °C for later observation at different exposure times.

## RESULTS

### Fourier transform-infrared spectroscopy

The results of the FT-IR analysis revealed the presence of the characteristic functional groups of the molecules constituting the films: chitosan, starch, and roselle extract. The signal between 3000 and 3500 $cm^{-1}$ showed stretching of O-H groups, while asymmetric and symmetric stretching of the aliphatic region ($CH_3$ and $CH_2$) were observed in 2800–2900 $cm^{-1}$, and stretching of C-O groups from C-OH and C-O-C was found in 900–1100 $cm^{-1}$. Finally, scissoring of $CH_2$, twisting of $CH_3$, and wagging of $CH_3$ were observed from 1400 to 1500 $cm^{-1}$ (see Fig. 1 and 2). Notably, an increase in chitosan concentration in the films evidenced changes in two signals: one due to the stretching of carbonyl groups (C=O, 1775 $cm^{-1}$) and the other corresponding to the bending of the amino group ($NH_2$, 1635 $cm^{-1}$) in the macromolecule. These two functional groups were more clearly observed when using 1.5% chitosan than a concentration of 1%. The functional groups corresponding to flavonoids in roselle extract (stretching of C=C, 1610 $cm^{-1}$) were overlapped in the scissoring bending vibration corresponding to the amino group ($NH_2$) in chitosan.

Fig. 3 and 4 show the vibration signals of flavonoids in roselle extract, and the signal at 1610 $cm^{-1}$ corresponds to C=C stretching while that in 700–800 $cm^{-1}$ refers to the bending of aromatic hydrogens. These vibration signals increased along with the concentration of roselle extract.

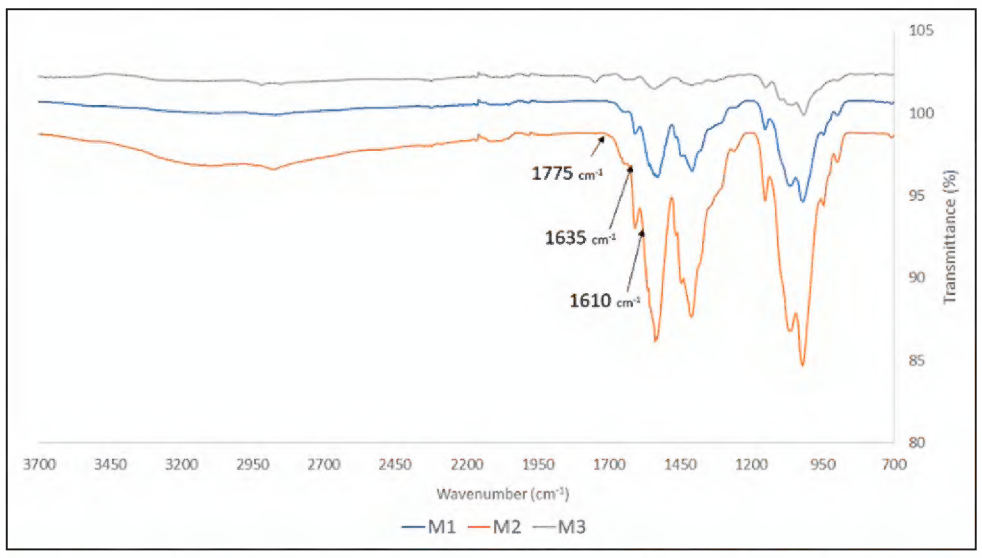



**Fig 1.** FT-IR analysis of samples M1 (1% chitosan), M2 (1% chitosan/0.5 mL roselle extract, and M3 (1% chitosan/1 mL roselle extract).

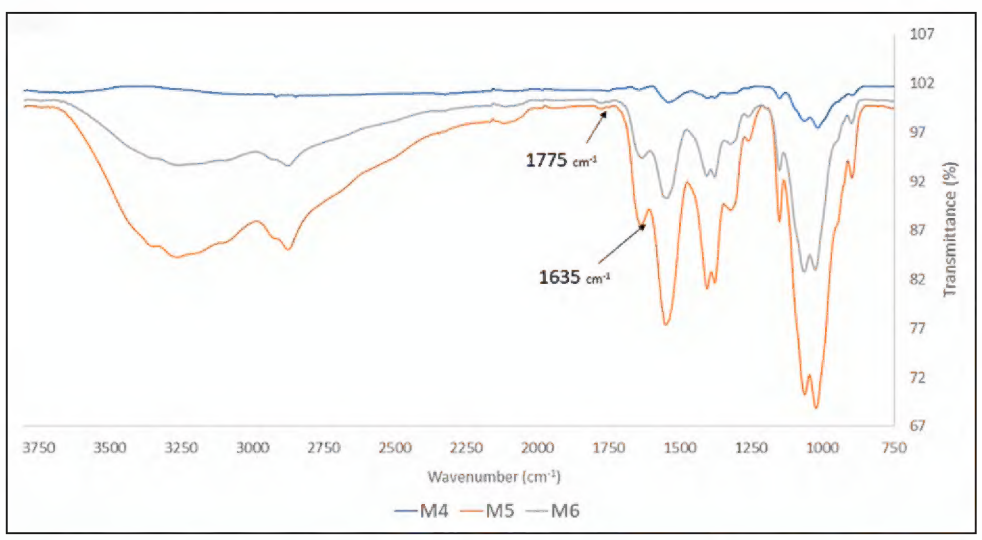



**Fig 2.** FT-IR analysis of samples M4 (1.5% chitosan), M5 (1.5% chitosan/0.5 mL roselle extract), and M6 (1.5% chitosan/1 mL roselle extract).

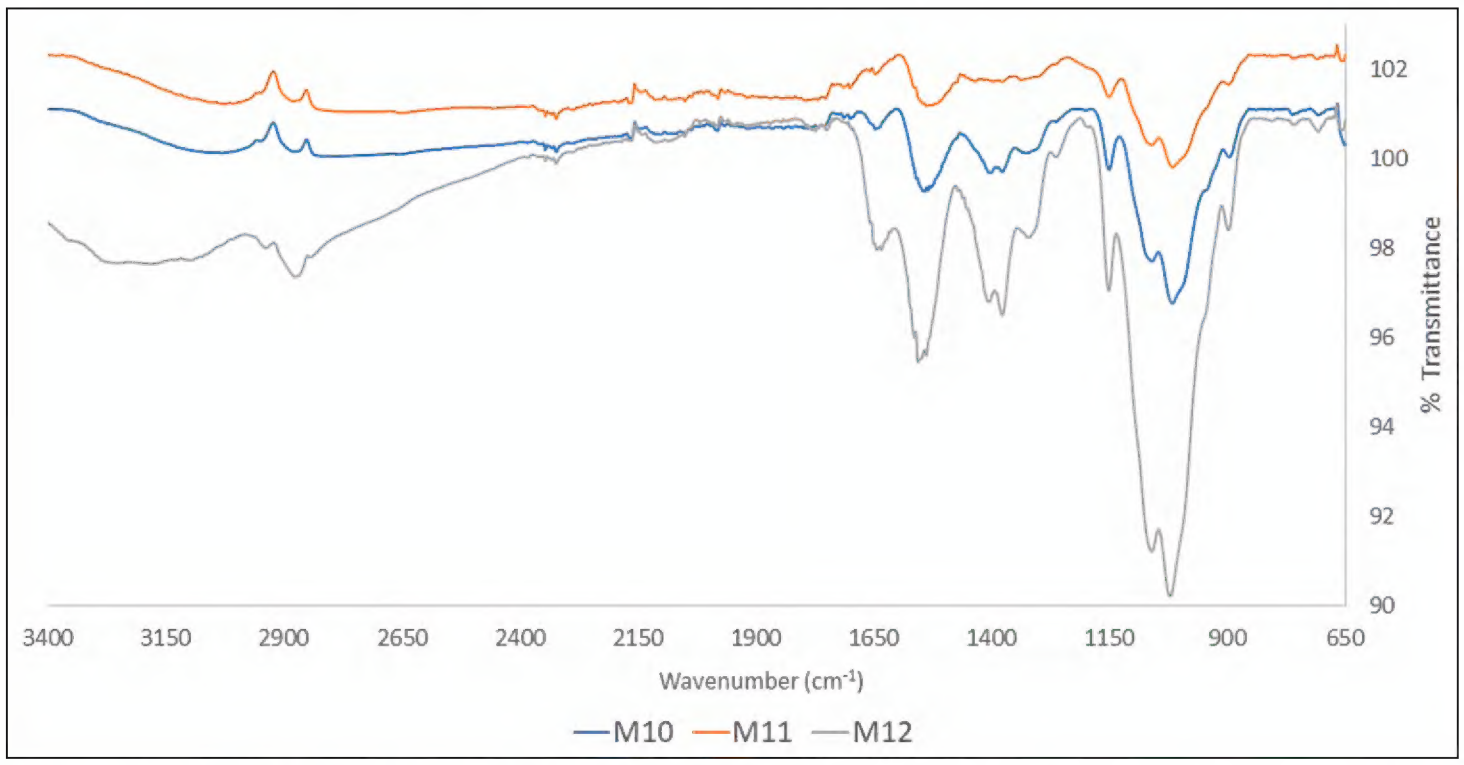



**Fig 3.** FT-IR analysis of samples M10 (1.5% chitosan/0.5% starch), M11 (1.5% chitosan/0.5% starch/0.5 mL roselle extract), and M12 (1.5% chitosan/0.5% starch/1 mL roselle extract).

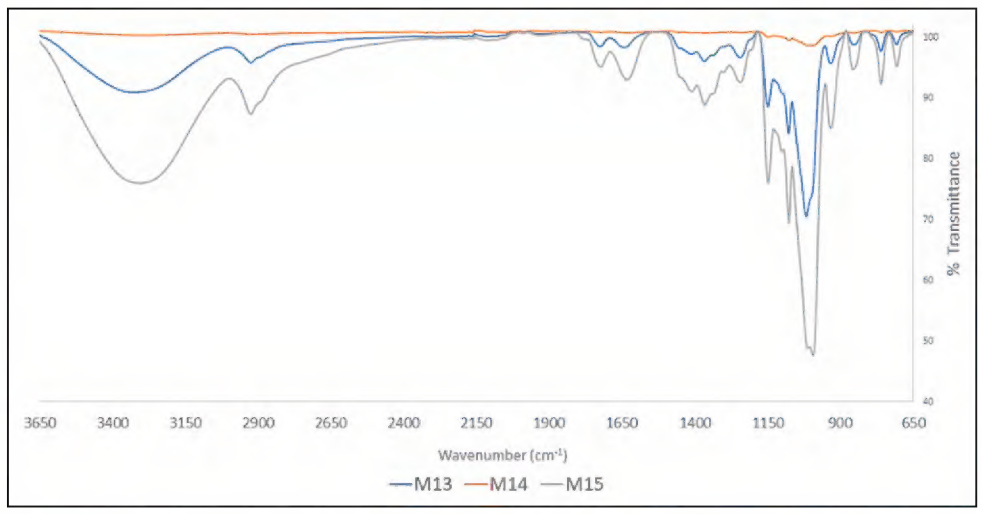



**Fig 4.** FT-IR analysis of samples M13 (0.5% starch), M14 (0.5% starch/0.5 mL roselle extract), and M15 (0.5% starch/1 mL roselle extract).

Additionally, the signals have been previously described by Bai et al. (1988) and Bertuzzi et al. (2012).

### Scanning electron microscopy

Fig. 5 presents the surface of the designed films. The images show that adding roselle extract to the samples produced no morphological alterations in the films, and there was a clear homogeneous phase.

### Contact angle

The analysis of the contact angle evidenced the presence of flavonoid groups in roselle extract since there was an increase in the contact angle of films M1–M3 and M4–M6 due to flavonoid aromatic rings. An increase in roselle extract concentration and the use of starch in M1–M13 proved the hydrophobic quality acquired by the films. Ceron-M et al. (2013) reported starch hydrophobicity in biofilm design.

### Thermogravimetric analysis

The TGA was carried out to identify the thermal stability of the films. To do so, a TGA and DSC calorimeter (TA Instruments, model SDT Q600 DSC-TGA Standard) was used. The samples weighed 3 mg and the maximum temperature was 770 °C.

**Table 2: Means of sample angles**

| Sample | Contact Angle ( Θ ) |
|---|---|
| M1 | 42.0 |
| M2 | 66.5 |
| M3 | 53.2 |
| M4 | 58.5 |
| M5 | 58.0 |
| M6 | 79.5 |
| M7 | 59.7 |
| M8 | 72.2 |
| M9 | 75.0 |
| M10 | 55.5 |
| M11 | 57.5 |
| M12 | 58.5 |
| M13 | 51.2 |
| M14 | 67.0 |
| M15 | 69.5 |

Table 3 shows the stages of film degradation. The first ranged between 124 and 188 °C and corresponded to the water adsorbed in the samples that also presented reduced moisture (M1–M3), confirming the results of increased contact angle. Similarly, starch contributed to hydrophobicity in the moisture reduction presented by samples M1 and M13.

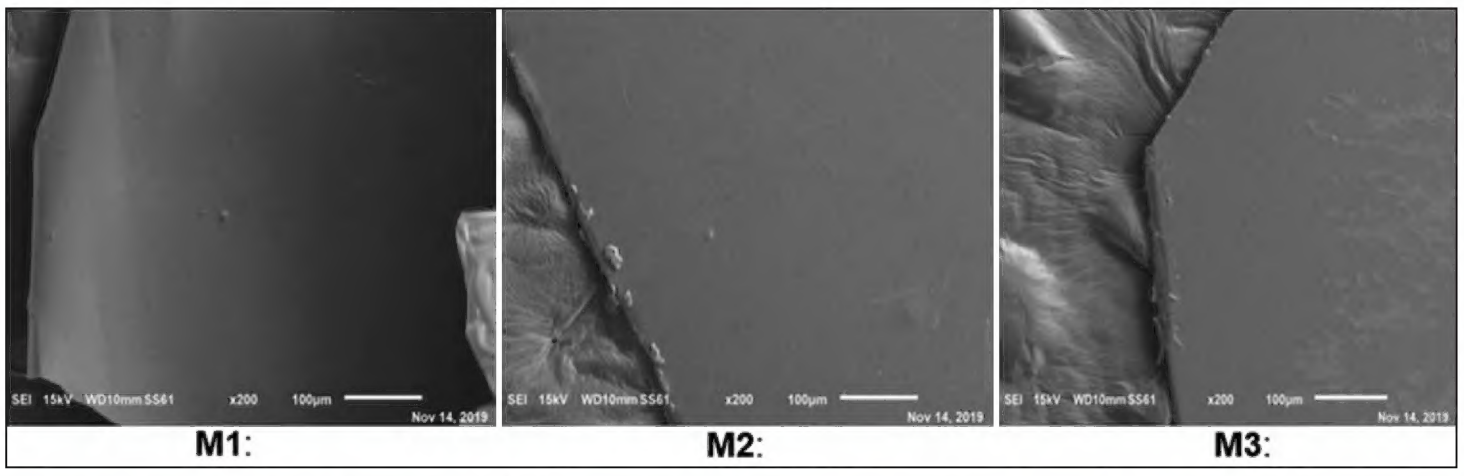



**Fig 5.** SEM analysis of samples M1 (1% chitosan), M2 (1% chitosan/0.5 mL roselle extract), M3 (1% chitosan/1 mL roselle extract). In Fig. 6, M10 presents a smooth and homogeneous surface of the films, and M13 evidences the surface with added starch where granules are observed. Finally, M14 shows a change in the formation of phases between starch and roselle extract.

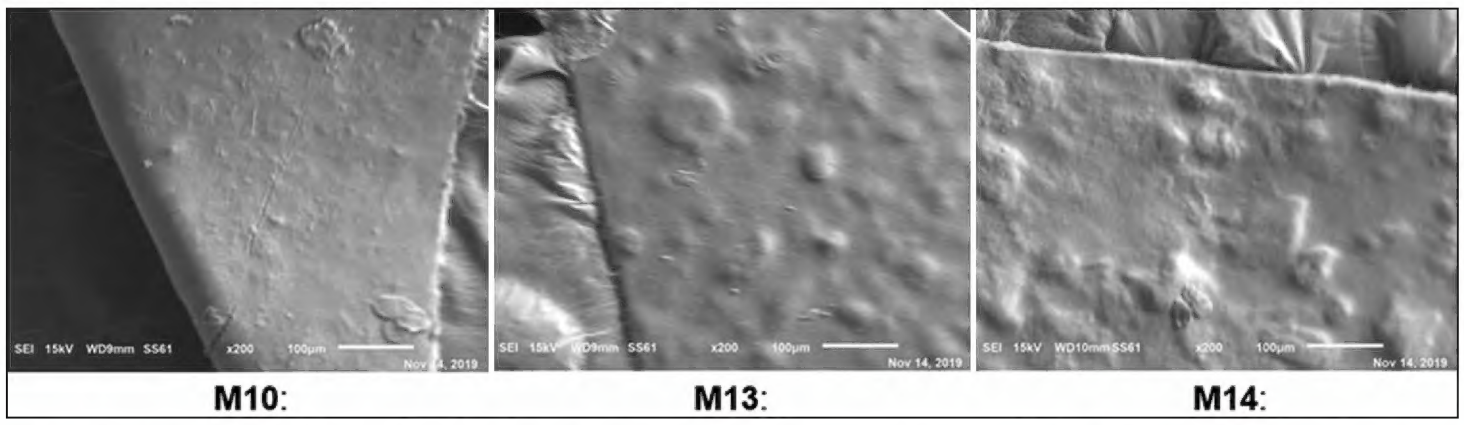



**Fig 6.** SEM analysis of samples M10 (1.5% chitosan/0.5% starch), M13 (0.5% starch), and M14 (0.5% starch/0.5 mL roselle extract).

Degradation in the second and third stages was 387–441 °C and 460–565 °C, respectively. Both corresponded to the degradation of the organic portion of roselle extract, starch, and chitosan matrix. In the second stage, chitosan increased the thermal stability of the samples to reach 441 °C. In the third stage, the roselle extract evidently affected thermal stability by reducing the degradation temperature to 512 °C. This was likely because the flavonoids in the extract can be degraded, breaking the aromatic structures, promoting the degradation of the material at a lower temperature. Lozano-Navarro et al. (2018) and Hugo Yves C. et al. (2019) have studied the stability generated by chitosan on the thermal properties of biofilms that use chitosan and starch.

**Table 3: Comparison (%) of weight and temperature between stages of the most representative samples**

| Sample | Stage I | | Stage II | | Stage III | |
|---|---|---|---|---|---|---|
| | % weight | T (°C) | % weight | T (°C) | % weight | T (°C) |
| M1 | 15 | 153 | 45 | 406 | 40 | 555 |
| M2 | 12 | 158 | 45 | 395 | 33 | 520 |
| M3 | 10 | 183 | 41 | 410 | 37 | 512 |
| | | | | | | |
| M2 | 12 | 158 | 45 | 395 | 33 | 520 |
| M5 | 15 | 178 | 44 | 441 | 39 | 565 |
| | | | | | | |
| M12 | 14 | 177 | 41 | 404 | 36 | 519 |
| M15 | 9 | 188 | 56 | 395 | 29 | 460 |
| | | | | | | |
| M13 | 10 | 176 | 63 | 414 | 16 | 484 |
| M14 | 8 | 124 | 52 | 387 | 24 | 477 |
| M15 | 9 | 188 | 56 | 395 | 29 | 460 |

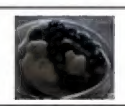
### Senescence analysis of blackberry fruits

Table 4 shows images of dip-coated blackberries at 0, 6, and 21 days when fruit senescence and the appearance of fungi were recorded. Once coated, the blackberries were kept in refrigeration at 7 °C and were monitored at different times to record the physical alteration of weight in the fruits for a period of 21 days.

When comparing samples M1 and M2, we observed that the use of 0.5 mL roselle extract in M2 contributed to preservation since the fruits showed no relevant physical alterations after 21 days. However, when the extract concentration increased to 1 mL, inhibition was not favored and fungi proliferated after some time, as shown in sample M3. Bacterial inhibition thanks to roselle extract was corroborated by Dwivedi, M. et al. (2020), Aydin, G. (2022), and Akarca, G. (2019).

It must be considered that chitosan confers antibacterial properties to films thanks to the amino groups in its structure, as the flavonoids in roselle extract do. When comparing samples M5 and M6, which contained 1.5% chitosan and 1 mL roselle extract (M6), we observed an inhibitory effect on day 21 of study and monitoring. In studies carried out by other authors, the antibacterial influence of the hibiscus psadariffa extract as a film on strawberry fruits has been observed (Estrada-Giron et al, 2023).Samples M7–M15 included starch to take advantage of the thermoplastic characteristics of the biopolymer to provide the fruits with plasticity and prevent brittleness. These samples did not inhibit microorganism growth after 21 days; therefore, starch worked as a medium for microorganism proliferation. It must be considered that both chitosan and starch contribute to improving

**Table 4: Photographs of blackberry coating on different days and lost weight**

| Sample | Day 0 | Day 6 | Day 21 | % Weight loss at 21 days |
|---|---|---|---|---|
| M1 | | | | 34.05 |
| M2 | | | | 15.01 |
| M3 | | | | 32.67 |
| M4 | | | | 25.10 |
| M5 | | | | 33.32 |
| M6 | | | | 13.16 |
| M12 | | | | 32.71 |
| M13 | | |  | 21.27 |
| M14 | | | | 32.78 |
| M15 | | | | 20.59 |

antibacterial properties, as proven by the results of this work. Some works that only use chitosan as coating can extend the shelf life of fruits as Brazilian guava, with poor mechanical properties (Zárate-Moreno, J.C. et al., 2023).

Samples M2 and M6 showed the most promising results in terms of fruit preservation thanks to chitosan and roselle extract.

## CONCLUSIONS

This work consisted in producing and characterizing chitosan/starch/roselle (*hibiscus sabdariffa*) extract films used when dip coating fruits (blackberries) to extend their shelf life thanks to the coating properties. It must be noted that the state-of-the-art literature reports no works that apply a chitosan matrix with starch and roselle extract as loads, which can contribute to the production of films that preserve fruits. The results obtained demonstrated the successful formation of a biofilm coating blackberries. The biopolymers in the coating deposited elicited a better response to the antimicrobial properties, and increased shelf life of the fruit four-fold, between 6 and 21 days. The study involved variables as chitosan, roselle extract, and starch concentrations. The use of a higher chitosan concentration at 1.5% contributed to increasing the thermal stability of the biofilm from 520 to 565 °C, as proven by the thermogravimetric analysis. In addition, there was antimicrobial inhibition due to an increase in secondary interactions between hydrogen bonds and the microbial properties of chitosan. The FT-IR and contact angle analyses evidenced the chemical interactions produced to favor the hydrophobic quality of the biofilms obtained because of the presence of chitosan functional acetyl and amino groups, starch OH groups and roselle flavonoids. The increase in roselle extract concentration, which reached 1 mL, did not favor microbial inhibition, as expected from the antioxidant effect. This was likely because of the phenolic resonance structures formed in the extract. The coated fruits corresponding to samples M2 and M6 showed microbial inhibition up to day 21 of shelf life, proving the technological contribution to fruit preservation. The aims of this research were satisfactorily achieved; still, there are certain suggestions for further works. It is recommended to coat climacteric fruits if possible to evaluate the behavior of the coating regarding breathing rate and gas exchange, as ethylene, in the fruits. It is also advised to use this method when coating meat products, especially chicken. Our research group began working on this application and, although the research activities had to be suspended due to the COVID pandemic, we have observed the results are better than those reported in the literature.

## CONFLICT OF INTEREST

There are no conflicts of interest between authors or participating institutions.

## ACKNOWLEDGMENTS

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### Authors' contributions

All the authors included in this article contributed equally to the work and their contributions were essential for the completion and preparation of this article. Dr. Rene Salgado Delgado and Dr. Alfredo Olarte Paredes and Dra. Areli Marlen Salgado Delgado are responsible for directing the project and for writing this article. Dra. Teresa Lopez Lara and Juan Bosco Hernandez Zaragoza was responsible for monitoring the experimental work. Dr. Edgar Garcia Hernandez and Dr. Rene Salgado Delgado served as advisors for the writing of the article and the interpretation of the data, while Ing. Adaia Ramirez Contreras headed the experimental work of the project in the laboratory and was involved in the writing of this article.

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